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Optimization of Short and Long Term Storage Duration for *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) at Low Temperatures*

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Abstract.- Efficient storage of the biological control agent is critical for commercial production. The purpose of this study is to assess the suitable storage temperature and duration for an important egg parasitoid, *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae). The fitness life parameters of *T. chilonis* such as percent emergence, percent parasitism and adult longevity were investigated under laboratory conditions after stored at six constant temperatures (6, 8, 10, 12, 14 and 16°C) for various storage durations (5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 days) in incubators. Highest (96.6%) *T. chilonis* emergence recorded at 10°C after 5 days storage and was similar to control (97.4%). However, emergence was reduced up to 22.8% at 10°C after 90 days storage. The parasitism was highest (97.4%) at 10°C after being kept for 5 days storage and was decreased to 42.2% at 10°C when stored for 90 days at same temperature. Adult longevity was decreased from 6.3 to 3.0 days when stored at 10°C from 5 to 90 days respectively. At 6°C, *T. chilonis* development was completed only after 25 days storage whereas, at 12, 14 and 16°C conditions, development were completed during storage hence these temperatures proved for short term storage. It was evident that, storage at 10°C was very conducive for life parameters of *T. chilonis* to get short and long term storage.

Key words: Low temperature, storage duration, *Trichogramma chilonis*.

INTRODUCTION

The egg parasitoid, *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) a minute wasp from a group of insects of great importance to biological control, is a natural enemy of many harmful lepidopterous insect pests of crops and vegetables. It has been considered an important parasitoid for more than 100 years. Field trials with these parasitoids prior to 1975 were intended to control lepidopterous pests of sugarcane and corn. Since 1975 *Trichogramma* has been used against the lepidopterous pests of cotton, cabbage, apple and tomato etc. (Smith, 1996). *Trichogramma* species is one of the most widely used biological control agent due to its easy rearing in insectaries and vigorous parasitism on eggs of target hosts. It has colossal abilities to parasitize the eggs of bollworms in cotton (King *et al*., 1984; Ahmad *et al*., 2001). This wasp has been shown to be very beneficial as a biological control agent in cotton and has a great potential to control cotton bollworms as the part of integrated pest management strategy (Ahmad *et al*., 1998).

Insects experience fluctuating temperatures in the natural environment and have evolved different adaptations to temperature extremes. An efficient biological control programme with *Trichogramma* needs a selection of strains in order to chose the most efficient one for use against a given target pest in a given set of environmental conditions (Hassan, 1994). By understanding the limits of storing parasitoids, *T. chilonis* can be made available all year around for field release to distant places from the insectaries and also provide parasitoids for cultures when they are not in demand. Effective storage can also provides the availability of stocks in mass rearing insectaries for research and field releases (Greenberg *et al*., 1996). Temperature directly influences the developmental period of parasitoids and the previous investigators have determined the effective low temperature on storage duration of *Trichogramma* species to prolong its shelf life to retain viability (Stinner *et al*., 1974; Jalali and Singh, 1992; Krishnamoorthy and Mani,

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1999; Hoffmann *et al*., 2001; Nadeem and Hamed, 2008). Lower and upper temperature thresholds for *Trichogramma* species are 9 and 36^{o}C, respectively (Kot, 1979). Kalyebi *et al*. (2004) compared parasitism rate by trichogrammatid egg parasitoids at six temperatures (10, 15, 20, 25, 30 and 35^{o}C) and two relative humidity levels (40-50% and 70-80%). Tezze and Botto (2003) has carried out research on new discovered species including *T. nerudai* Pintureau and *T. nerudai* Gerding to study the cold storage effects on quality. Pupae of *T. nerudai* were stored for 25, 50, 75, 100, 125 and 150 days at 4±1^{o}C with RH 75±5% in complete darkness. The fitness parameters of *T. nerudai* were affected after 50 days storage. Pitcher *et al*. (2002) have reported an efficient production method of *T. ostriniae* reared on moth eggs, *Sitotroga cerealella* (Olivier) and stored at 6, 9, 12, 15 and 24^{o}C for 8 weeks after parasitism. At 15^{o}C, emergence was completed in two weeks as compared to storage of 4 and 6 weeks at 9 and 12^{o}C, respectively. The percent emergence declined gradually after stored at 6^{o}C. Rate of parasitism by stored *Trichogramma* was similar to control after 2 to 4 weeks storage at 9 and 12^{o}C but parasitism declined when storage period increased to more than 4 weeks and evaluated 9^{o}C as the most suitable storage temperature. The relationship between temperature and storage is presented in the context of work on strains of *Trichogramma* other than *T. chilonis*, the most important local specie. We attempted in the present study to find out the optimum low temperature and storage duration effects on *T. chilonis* quality parameters such as percent emergence, percent parasitism and adult longevity.

MATERIALS AND METHODS

The study was conducted in mass rearing laboratories of beneficial insects at Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan, to find out the optimum storage temperature and storage duration of parasitoids, *T. chilonis* at its pupal stage in incubators. Experiment was carried out on parasitoid colony maintained on eggs of *Sitotroga cerealella* (Olivier) reared on wheat grains. *Trichogramma chilonis* was reared in glass vessels as described by Morison (1970). Two hundred host eggs were pasted on paper card strips and exposed for 24 hours to one day old parasitoids confined in glass vessels. Honey solution (10%) on paper strip was provided to parasitoid as an adult diet inside the glass vessels. After 24 hours exposure to parasitoids, the host eggs on strip referred as parasitized eggs were taken out from glass vessels and kept in petri dishes under standard laboratory conditions i.e., 25±2^{o}C and 65±5% RH at 14:10 light and dark period until parasitoids reached the pupal stage after seven days of parasitism.

Pupae of *T. chilonis* were kept at six constant temperatures of 6, 8, 10, 12, 14 and 16°C for various storage durations *i.e.* 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 days in complete darkness by making completely randomized design using three replication for each treatment of respective storage temperature and duration in incubators. After each designated storage duration, parasitoid strips were taken out from the incubator and were placed in standard laboratory conditions to allow parasitoid adult emergence. Data on fitness parameters of adult parasitoids as percent emergence, percent parasitism and adult longevity (days) were recorded and compared to un stored control at standard laboratory conditions. Data were statistically analyzed by using MSTATC software programme (Steel *et al*., 1997) and Duncan's multiple range test (DMRT) was applied to find the significance of results.

RESULTS AND DISCUSSION

Percent emergence of T. chilonis

At 5 days storage, the highest (96.6%) emergence was observed from *T. chilonis* parasitoids after held at 10^{o}C storage and it was near to control (97.4%), followed by 94.1, 90.7, 89.9, 88.5 and 64.7% held at 8, 12, 14, 16 and 6^{o}C, respectively (Table I). Similar trends of parasitoids were observed at 10, 8, 14, 12, 16 and 6^{o}C after 10 days storage with emergence of 94.3, 91.6, 88.6, 88.4, 86.8 and 49.2%, respectively. Highest and lowest emergence was observed at 10 and 6^{o}C, respectively after 15, 20 and 25 days storing period. At 6^{o}C, the parasitoids did not survive after 25 days storage due to continuous low temperature, while at 16^{o}C, the parasitoids complete their development due to moderate temperature and emerged after 25 days. Similarly at 14 and 12°C, parasitoids complete their development and emerged out after 30 and 40 days storage, respectively. Parasitoids emergence was recorded at 8 and 10°C up to 90 days storage conditions. So here it is clear that for short term storage, the parasitoids emergence was best up to 25 days at 10°C and parasitoid can be used for field releases. Whereas prolonged storage gradually decrease the percent emergence after 90 days storage and gave only 21.0 and 22.8% emergence at 8 and 10°C respectively. In our findings, the adult emergence of *T. chilonis* at 12°C was 84.8% after 15 days of storage and is similar to results reported by Pitcher *et al*. (2002) who have got 90.7% emergence of *T. ostriniae* parasitoids at same temperature and days. At 6°C storage, parasitoids did not survive after 25 days whereas at 16°C, parasitoids completed development and emerged during storage and our findings disagreed to the results reported by Pitcher *et al*. (2002) that at 15°C, parasitoids (*T. ostriniae*) did not survive more than 2 weeks. Tezze and Botto (2003) reported that *T. nerudai* stored at 4°C were greatly affected from 50 days to onward in storage but in our work, *T. chilonis* emergence was completed in 25 days at 6°C, indicating that duration in storage varies among the different *Trichogramma* species. Our results are similar to the findings of Zhu *et al*. (1992) who reported that *T. evanescens* were not viable for very long after stored at low temperature. The emergence of *T. chilonis* from 88.5 to 85.9%, obtained after 5 to 25 days of storage in present investigation are also similar to results reported by Harrison *et al*. (1985) who observed 88.0% emergence at 15°C for *T. exiguum.*

Percent parasitism by T. chilonis

The highest (97.4%) parasitism was attained from, the *T. chilonis* parasitoids held at 10°C after 5 days storage, which is close to values in the control treatment with 97.8% (Table II). Parasitism by parasitoid held at 8, 14, 12, 16 and 6°C were 96.3, 95.2, 95.1, 92.5 and 92.4% respectively. After 10 days storage the parasitism was observed 96.3, 95.7, 94.3, 92.6, 90.7 and 91.4% at 10, 8, 12, 14, 16 and 6°C respectively. Temperature of 6°C did not provide storage more than 25 days due to continuous cold effect and hence did not obtain the parameter of percent parasitism. While at 16°C the parasitoids complete their development during storage due to suitable temperature for development. At 14°C, after 30 days storage, percent parasitism by parasitoids was zero due to completion of their development. At 10°C from 50 to 90 days storage, parasitoids gave 72.5-42.2% parasitism. Here it is clear that for short term storage, the parasitoids gave best parasitism for 25 days at 10°C and can be viable for field releases. Whereas, prolonged storage gradually decreased the parasitism after 90 days storage with 38.7 and 42.2% at 8 and 10°C respectively. Our results on *T. chilonis* parasitism agreed to the investigations of Pitcher *et al*. (2002) who have reported that parasitism by stored *T. ostriniae* was not affected adversely at 9°C for 30 days storage but declined at 12°C for 30 days. Our findings are also agreed with results reported by Harrison *et al*. (1985) who got 83.6% parasitism at 15°C for *T. exiguum* vs. 89.9% at 14°C in the present work.

Longevity of T. chilonis *adults*

Developmental period of *T. chilonis* was prolonged to 6.3 days after held at 10°C followed by 6.3, 5.6, 4.6 and 4.6 days at 8, 6, 12, and 14°C respectively (Table III). Duration of development reduced up to 4 days at 16°C after 5 day storage. At 6, 8, 10, 12, 14 and 16°C, storage after 10 days showed the similar trend of adult longevity 5.3, 6.3, 6.3 4.6, 4.6 and 4.3 days, respectively. The prolonged and reduced adult longevity was observed at 10 and 16°C respectively after stored for 15, 20, and 25 days. Temperature of 6 and 16°C proved effective only for 25 days storage but the further observations on adult longevity was not possible due to unavailability of parasitoids owing to continuous lower and high temperature, respectively. At 16°C the parasitoids completed their development during storage. Similarly parasitoids emerged during storage at 14 and 12°C. Adult longevity gradually decreased as the storage duration increased from 5-90 days for 6-16°C. Prolonged storage gradually decrease the adult longevity and after 90 days storage, parasitoids survived only for 3 days at 10°C. Our findings on decreased adult longevity at 8°C for long term storage are agreed to a study reported by Özder

Table I.- Percent emergence of *T. chilonis* after stored at various storage conditions.

Temp.	Storage duration (Days)											
	5	10	15	20	25	30	40	50	60	70	80	90
6°C	64.7±1.20d	49.2±2.40c	45.2±1.74c	23.5±1.73b	08.0±1.42c	-	-	-	-	-	-	-
8°C	94.1±0.87ab	91.6±2.09ab	89.7±1.10ab	88.2±2.06a	87.5±1.53a	82.5±0.82b	78.6±1.13ab	77.6±1.91a	72.9±1.27a	62.4±1.44a	47.6±1.16a	21.0±0.80a
10°C	96.6±1.46a	94.3±1.12 a	91.8±1.04a	88.7±2.27a	84.8±1.77ab	85.5±0.57a	80.4±0.42a	78.8±0.73a	76.6±0.50a	70.6±0.48b	45.2±2.07a	22.8±1.58a
12°C	90.7±1.46bc	88.4±1.58ab	84.8±2.43b	86.0±0.90a	80.2±2.28b	79.3±0.57c	77.6±0.61b	-	-	-	-	-
14°C	89.9±1.47c	88.6±1.33ab	84.0±2.47b	85.5±1.86a	83.7±0.76ab	80.3±0.69c	-	-	-	-	-	-
16°C	88.5±0.82c	86.8±1.87b	88.6±1.65ab	87.8±1.19a	85.9±1.62ab	-	-	-	-	-	-	-
Control	97.4 ±0.52a											

Means (± SE; n=3) sharing same alphabets are statistically non significant (P<0.05).

Table II.- Percent parasitism of *T. chilonis* after stored at various storage conditions.

Temp.	Storage duration (Days)											
	5	10	15	20	25	30	40	50	60	70	80	90
6°C	92.4±1.32b	91.4±0.65c	82.4±0.32c	80.3±0.60c	48.5±1.16d	-	-	-	-	-	-	-
8°C	96.3±0.45a	95.7±0.31a	92.6±1.07a	91.0±0.69a	88.6±1.22b	80.2±0.49b	78.1±0.64b	74.0±0.97a	72.7±0.78a	69.3±1.01a	59.6±1.21a	38.7±1.69a
10°C	97.4±0.81a	96.3±0.60a	91.9±1.33a	92.3±1.18a	91.5±0.41a	88.3±0.72a	84.1±0.83a	72.5±1.05a	74.5±1.10a	72.6±0.60a	63.4±1.32a	42.2±4.19a
12°C	95.1±0.52a	94.3±0.37ab	91.4±0.72a	90.1±0.96a	86.4±0.83b	81.1±0.78b	71.2±0.75c	-	-	-	-	-
14°C	95.2±0.49a	92.6±1.19bc	90.0±0.64a	85.4±0.57b	82.3±0.60c	80.5±0.72b	-	-	-	-	-	-
16°C	92.5±0.34b	90.7±1.41c	84.1±1.99b	82.9±1.12b	80.2±0.69c	-	-	-	-	-	-	-
Control	97.8±0.40 a											

Means (± SE; n=3) sharing same alphabets are statistically non significant (P<0.05).

Table III.- Adult longevity (days) of *T. chilonis* after stored at various storage conditions.

Temp.	Storage duration (Days)											
	5	10	15	20	25	30	40	50	60	70	80	90
6°C	5.6±0.33a	5.3±0.33abc	6.0±0.00a	5.6±0.33a	5.3±0.33bc	-	-	-	-	-	-	-
8°C	6.3±0.33a	6.3±0.33ab	6.3±0.33a	5.6±0.33a	6.3±0.00a	5.3±0.33ab	4.6±0.33a	3.3±0.33a	3.6±0.33a	3.6±0.33a	2.6±0.33a	2.6±0.33a
10°C	6.3±0.33a	6.3±0.00a	6.6±0.00a	5.3±0.33ab	5.6±0.33ab	5.6±0.33a	3.6±0.33a	3.6±0.33a	3.3±0.33a	3.3±0.33a	2.3±0.33a	3.0±0.33a
12°C	4.6±0.33ab	4.3±0.33c	5.0±0.57ab	4.3±0.33b	5.6±0.33ab	4.6±0.33ab	3.6±0.33a	-	-	-	-	-
14°C	4.6±0.33ab	4.6±0.33bc	6.0±0.00a	4.6±0.33ab	5.3±0.33abc	4.3±0.33b	-	-	-	-	-	-
16°C	4.0±0.57b	4.6±0.33bc	5.0±0.33b	4.3±0.33b	4.6±0.33bc	-	-	-	-	-	-	-
Control	5.0±0.57ab											

Means (± SE; n=3) sharing same alphabets are statistically non significant (P<0.05).

(2004) where adult longevity of egg parasitoid, *T. cacoeciae* were also decreased after 31 days storage at 8°C.

CONCLUSIONS

It is concluded from the present study that 10°C is the most effective temperature for storage of *T. chilonis* parasitoids, where highest percent emergence, percent parasitism and adult longevity has been recorded. Moreover this temperature suited both for the long and short term storage that ensures the availability of parasitoids in insectaries for research and field releases.

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